

STUDY PROTOCOL

I. STUDY TITLE: Remote Ischemic Conditioning (RIC) in Recipients of Brain Death Donor Livers – A Feasibility and Safety Study

II. STUDY GOALS AND OBJECTIVES:

- 1) To determine the feasibility of lower limb ischemia-induced RIC immediately before, during and in the first four post-operative days in recipients of livers from brain death donors (BDD).
- 2) To obtain preliminary evidence that RIC is safe in recipients of BDD livers.
- 3) To obtain preliminary evidence that RIC decreases post-operative complications in recipients of BDD livers.

III. BACKGROUND:

Orthotopic liver transplantation (OLT) is associated with a very high risk of complications. In a recent multi-center study of 450 patients, 79% had at least one complication and 63% had severe (\geq Clavien-Dindo grade III) complications. The number and severity of complications are associated with death within 30 days, hospital length of stay, graft and patient survival.¹ Infections are the most common group of complications, followed by pulmonary, renal and liver graft dysfunction.¹

Prolonged respiratory insufficiency (PRI), often defined in the literature as requirement for mechanical ventilation for >48 hours or reintubation after initial extubation, occurs in 17-42% of liver recipients.^{2,3} As in other surgical populations,⁴⁻⁶ prolonged need for mechanical ventilation in liver recipients increases resource utilization, prolongs ICU and hospital length of stay, and increases mortality.^{2,3}

In a recent report, acute kidney injury (AKI) within the first 72 hours after liver transplantation was reported in 52% of patients.⁷ Contributory factors include medical comorbidities, hepatorenal syndrome, intra-operative blood loss and/or hypotension, cytokine release secondary to liver reperfusion, post-operative complications such as bleeding and sepsis, and the use of calcineurin inhibitors. The development of post-operative AKI in liver transplant recipients is predictive of moderate and severe chronic kidney disease (CKD), dialysis dependence at 3 months and 1 year post-transplant, and decreased five year graft survival.⁷

Early allograft dysfunction (EAD) is another important complication after OLT. Its incidence ranges from 20-30% but rates as high as 52% have been reported.⁸ Patients with EAD have several fold higher risk of graft loss and death in the first 6 months after transplant and utilize a greater amount of medical resources.⁹

Thus, interventions that decrease these complications after OLT are likely to improve clinical outcomes.

Remote ischemic conditioning is an innate biological phenomenon wherein a brief single or repetitive ischemic stimulus in an organ or tissue such as skeletal muscle induce protection in remote/distant organs against ischemia and other noxious stimuli.¹⁰ This effect can be induced by inflating a pneumatic tourniquet on a leg or arm for a few minutes (usually 5-10) and subsequently deflating to

allow reperfusion. This process is usually repeated 3-4 times to ensure an adequate dose of the conditioning stimulus. The conditioning stimulus could be applied before (Preconditioning), concurrent with (Perconditioning), or soon after the index noxious/ischemic insult (Postconditioning).^{11,12} In a recent multi-center clinical trial of RIC in brain death donors, lower limb ischemia-induced preconditioning led to a slight decrease in liver reperfusion injury when cold ischemia was ≥ 5 hours. Furthermore, RIC improved 6-month kidney allograft survival (pending publication).

A reduction in complications after organ transplantation is more likely to occur with the use of limb ischemia-induced RIC in recipients in comparison to its use in donors. First, RIC has the potential to ameliorate graft reperfusion injury. Second, RIC may protect many other recipient organs – lungs, kidneys, heart and brain –from the deleterious effects of hypoperfusion during surgery and other injurious pathophysiological events in the early postoperative period. Third, conditioning could be induced in the recipient at multiple time points (pre-, per-, and postconditioning) with the potential to have an additive effect.

Several prospective randomized clinical trials of RIC have been reported in patients undergoing various types of surgery including renal transplantation.¹³⁻¹⁶ In all except one,¹⁶ RIC was applied under general anesthesia. Therefore, the potential to cause any significant discomfort/pain during conditioning did not exist. Although other studies have demonstrated the tolerability of RIC in awake patients¹⁶ and in healthy volunteers,¹⁷ whether patients that have undergone major abdominal surgery such as liver transplant would tolerate daily transient limb ischemia is not known.¹⁸⁻²⁰ In prospective randomized clinical trials to date, RIC did not lead to any deleterious clinical effects. Hence our objective in this study is to assess the feasibility, patient acceptance, and safety of RIC in liver recipients. In addition, we will obtain data on posttransplant complications. Information obtained from this study will help guide the design of a future randomized, controlled trial to test the benefit of RIC in liver recipients.

IV. HYPOTHESES:

In recipients of BDD livers, intra- and postoperative remote ischemic conditioning is feasible. The discomfort/pain associated with postoperative conditioning will not lead to withdrawal of participation by alert patients. Remote ischemic conditioning is safe in recipients of BDD livers.

V. STUDY DESIGN:

A singlecenter, single cohort, prospective, non-randomized study of RIC will be conducted in adult recipients of livers from BDD. All participants will receive RIC during transplant and the initial four post-transplant days. RIC will be induced at two time points during transplant; first after induction of anesthesia but before commencing surgery and the second at the conclusion of the procedure. After transplantation, RIC will be applied daily during the first four consecutive postoperative days. Each RIC intervention will comprise three cycles of 5 minutes of inflation followed by 5 minutes of deflation of a pneumatic tourniquet placed in mid-thigh. Lower limb ischemia will be confirmed with bedside assessment of lower extremity Doppler signals before and during tourniquet inflation.

The purpose of the study will be to assess the feasibility of conducting a prospective, randomized, controlled trial of RIC in OLT recipients. Outcomes, as described below (Section VII. 3-4), will focus on patient acceptance and tolerability of the RIC interventions. Other outcomes will focus on the feasibility of patient recruitment, enrollment, data collection, and adequate followup.

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Preliminary data on the efficacy of RIC in OLT will be obtained from comparison with age-, sex-, and MELD-matched historic controls closest in time era to the subjects in this trial. Depending on the number of control subjects available we will match each case with 1-4 controls (matched within each center). It is expected that such a comparison would yield preliminary data which would improve design of future larger, randomized trial.

VI. STUDY POPULATION:

Adult (≥ 18 years of age) recipients of BDD livers at University Hospital in Newark, NJ.

VI. 1. Inclusion Criteria

- 1) Adults (≥ 18 years of age) with acute and chronic liver failure requiring liver transplants or patients undergoing transplantation for hepatocellular carcinoma.
- 2) Both sexes
- 3) Written consent to participate in the study

VI. 2. Exclusion Criteria

- 1) < 18 years of age
- 2) Recipients of split livers
- 3) Retransplantation
- 4) Recipients of livers combined with other organs
- 5) Recipients of livers from cardiac death donors
- 6) Lower extremity amputees
- 7) History of peripheral vascular disease
- 8) Patients taking sulfonylurea anti-diabetic agents at the time of transplant*
- 9) Patients taking nitrates at the time of transplant*
- 10) Body mass index > 45
- 11) Pregnant patients
- 12) Patients in whom complete lower extremity ischemia is not achieved despite maximum tourniquet inflation to 250 mmHg during the first intervention
- 13) Patients with lower extremity paralysis

* - see Appendix A for comprehensive list of drugs that fulfill exclusion criteria.

Inclusion of both sexes and all ethnic groups will provide a broad representation of all segments of the potential liver recipient pool. Children are excluded because our center does not perform liver transplantation in patients under age 18. Due to significant differences in the complications burden, patients undergoing retransplantation, as well as those receiving multiple organs and those receiving cardiac death donor organs are excluded. In addition, the numbers of such patients are too small to achieve successful randomization in future studies. Lower extremity amputees are excluded due to lack of adequate muscle. Patients with history of peripheral vascular disease and patients taking sulfonylurea agents and/or nitrates are excluded due to the potential for either abrogation or mimicking of conditioning benefit.²¹ Severely obese patients are excluded due to concerns regarding incomplete lower extremity ischemia from tourniquet inflation. Patients with lower extremity paralysis will be unable to report pain from the RIC intervention and are therefore excluded.

VII. RESEARCH METHODS:

VII. 1. Recruitment and Enrollment: The candidates completing an evaluation for liver transplantation, and are either accepted or are about to be accepted for liver transplant listing at University Hospital in Newark, NJ will be screened for eligibility. Eligible subjects will be approached in the transplant clinic at each site's respective outpatient transplant clinics for possible participation in the study. Eligible subjects that are inpatients at University Hospital will be approached while still hospitalized. Written informed consent will be obtained either from the subject or from the legally authorized representative at the time of recruitment. For patients who do not speak English, informed consent will be obtained with the help of a licensed interpreter; however, due to limitations in funding, we are unable to use non-English informed consent documents. Subjects will be enrolled into the study only when the donor liver is deemed suitable for transplantation, and a decision is made to proceed with transplantation.

VII. 2. RIC Intervention and Perioperative Care: The RIC intervention will occur at six time points. The initial intervention would occur in the left thigh after induction of anesthesia but before commencing skin incision for the transplant. The second intervention would occur in the left thigh at the end of the procedure. Starting the day after surgery, RIC will be repeated daily during the first four postoperative days. Each of those interventions will occur between 8am-10am to accommodate logistic practicalities. The first of these four postoperative interventions would occur on post-operative day 1, with post-operative day 0 defined as the calendar date of completion of the procedure. The post-operative interventions would be applied on alternate lower limbs commencing with the right.

Each RIC intervention would comprise three cycles. Each cycle would consist of 5 minutes of inflation of a pneumatic tourniquet placed in mid-thigh followed by deflation of the cuff for 5 minutes. Tourniquet pressures will be 250 mmHg for the pre- and intra-operative interventions. For the postoperative interventions, the tourniquet inflation pressure will be 50 mmHg above the patient's systolic blood pressure. During tourniquet inflation, Doppler signals in the patient's foot will be assessed. If there is incomplete ischemia, the tourniquet pressure will be gradually increased 10 mmHg at a time until ischemia is achieved up to a maximum of 250 mmHg. If a communicative patient expresses excess pain from tourniquet inflation at any point, and asks that the intervention be stopped, the RIC intervention will be terminated. The subject will not receive any further RIC interventions. The rationale for using a higher pressure during the two interventions in the operating room is based on our empiric observations that the systolic blood fluctuates considerably in the operating room in comparison to the postoperative period.

The remainder of perioperative and post-transplant care will be as per standard of care. Of note, however, propofol use will be avoided during induction and maintenance of anesthesia, as studies have shown that propofol may diminish the beneficial effects of RIC.¹⁵ Anesthetic induction using etomidate and maintenance with volatile agents will be the preferred regimen. Similarly, propofol use for post-operative sedation will also be discouraged, but ultimately its use will be at the discretion of the critical care team caring for the patient.

VII. 3. Primary Outcome: Proportion of enrolled liver recipients that complete all 6 RIC

interventions. In subjects that receive fewer than 6 interventions, we will report the number of interventions each received.

VII. 4. Secondary Outcomes:

- 1) Proportion of subjects screened who are found to be eligible, and the reasons for ineligibility.
- 2) Proportion of subjects eligible for the study who are successfully recruited (see Appendix B) for participation. Of those recruited, proportion of subjects who are successfully enrolled (see Appendix B), proportion who withdraw consent while awaiting transplantation, proportion who are unable to be enrolled due to logistic difficulties, proportion who die awaiting liver transplantation and proportion still waiting on the list at the end of the study.
- 3) Proportion of patients enrolled who complete follow up at 90 days.
- 4) Proportion of patients who withdraw consent from study participation after initial enrollment but before study completion.
- 5) Median intervention-related pain score during on each day of the post-operative intervention, in extubated patients who are able to communicate.
- 6) Logistical feasibility of RIC interventions at the stated time points.
- 7) Adverse events related to the RIC intervention:
 - Withdrawal of consent due to discomfort/pain in the lower extremity.
 - Any other adverse event considered by the primary clinical team to be related to RIC.
- 8) Early Allograft Dysfunction (EAD), defined as:
 - Aspartate Transaminase (AST) or Alanine Transaminase (ALT) > 2,000 U/L at any point within the first seven post-transplant days, or
 - Total Bilirubin (TB) \geq 10 mg/dL on postoperative day 7, or
 - International Normalized Ratio (INR) \geq 1.6 on postoperative day 7.⁹
- 9) Prolonged Respiratory Insufficiency (PRI), defined as:
 - Ventilator support for >2 postoperative days after transplant, or
 - Reintubation after extubation, within 7 days of transplant. Patients who require brief re-intubation for an endoscopic, radiologic, or surgical procedure would not be considered to have PRI if they are extubated within 2 days of the end of the procedure.
- 10) Acute Kidney Injury (AKI) stages 2 or 3 by KDIGO criteria. Criteria are as below:
 - Stage 2:
 - 2.0-2.9 fold rise in serum creatinine from baseline
 - Stage 3:
 - \geq 3.0 fold rise in serum creatinine from baseline, or
 - Serum creatinine of \geq 4.0 mg/dL, with an acute (<48 hours) increase of 0.3 mg/dL in serum creatinine or subacute (< 7 days) increase in serum creatinine of 0.5 mg/dL, or
 - Initiation of renal replacement therapy.²²

Of note, patients already receiving renal replacement therapy at the time of transplantation are, by definition, already in stage 3 AKI, and would be excluded from analysis of this outcome. Most patients with pre-operative creatinine of 3.6 mg/dL or greater would also likely have stage 3 AKI prior to transplant, and will also be excluded from analysis of this outcome. Urine output criteria for the assessment of AKI will not

be used due to the poor reliability of urine output measurement once patient leaves intensive care unit and after removal of Foley catheter.

- 11) In patients who are receiving dialysis pre-op, time to discontinuation of dialysis, if occurring within 90 days of transplantation.
- 12) Proportion of patients with Clavien-Dindo \geq grade III b complication, as well as number of such complications per patient.
- 13) Length of ICU and hospital stay post-transplant.
- 14) Patient quality of life at 90 days post-transplant as determined by World Health Organization Quality of Life-BREF (WHOQOL-BREF) survey, as well as response rates to surveys administered.
- 15) 90 day graft and patient survival.

To standardize count of postoperative days in this study, we will consider the calendar date of completion of the transplant as postoperative day 0.

VII. 5. Data Collection: Data regarding subject screening for eligibility, recruitment, and enrollment will be stored in an excel spreadsheet on a dedicated Rutgers server with password protection. Other clinical data will be entered electronically into case report forms via the online REDCaps software. Hard copy case report forms are also available as needed to facilitate the transfer of data to online software (see VII. 8).

Baseline recipient variables collected will include age, sex, ethnicity, height, weight, baseline medical characteristics, baseline laboratory values and care needs at transplant (whether hospitalized, in ICU, etc).

Baseline donor variables will include a unique donor ID, the United Network for Organ Sharing Network (UNOS) ID, age, sex, ethnicity, height, weight, baseline medical history and information related to medical status, pretransplant biopsy results, and cold ischemia time. Donor risk index (DRI) will be calculated.²³

Operative variables will include date and start of surgery, duration of surgery, timing of critical intra-operative events, warm ischemia time, blood loss and fluid replacement, technical aspects of the surgery, and information about the timing and type of biopsies taken (if any).

Variables related to outcomes will include admission date, transplant date, discharge date, intensive care unit days, total hospital days, post-transplant hospital days, duration of mechanical ventilation, relevant laboratory data and major complications (including death) identified as per Clavien-Dindo grading. 90 day outcome data will also be collected regarding patient's renal function, dialysis dependence, quality of life using WHOQOL-BREF, allograft survival, and patient survival.

Intervention-related variables will include date and time of each intervention, pressures used to achieve ischemia, patient's pain during the intervention, whether each intervention was completed or not, and whether there were any deviations from the protocol as described.

VII. 6. Case Report Forms (CRF)

An Excel spreadsheet (the enrollment tracking spreadsheet) will list all patients screened for study participation. This spreadsheet will be used to track study eligibility, recruitment, enrollment, and follow up. Reasons for ineligibility will be recorded. If a patient does not complete the study after recruitment, brief notes will be made to capture the reasons for discontinuation.

The REDCaps (research.njms.rutgers.edu/redcap/) electronic data capture software will serve as the primary means of data capture. A total of 8 forms will be utilized:

1. Recipient and donor baseline characteristics will be captured in CRF – 1 and 2, respectively. The donor's OPO record and excerpts of the recipient inpatient medical record will serve as the source documents.
2. Operative information will be captured in CRF – 3. Anesthesia records, as well as the patient's operative report from the recipient's medical record, will serve as the source documents.
3. Outcome-related information will be captured in CRFs – 4-6. Liver and lung outcome data will be captured in CRF 4, renal outcome data will be captured in CRF 5, and complication outcome data will be captured in CRF 6. The recipient's inpatient medical record will serve as the source document.
4. Intervention data will be captured in CRF 7. A hard-copy, paper worksheet version of CRF 7 has been created to facilitate intervention-related data capture, as the intervention related data will not be available in the patient's medical record. This will be filled out by the person performing the intervention while the intervention is in progress. Data will then be transferred to the electronic REDCaps software. Hard copies of the paper chart for CRF 7 will be discarded in a manner consistent with University Hospital protocol after data transfer and audit.
5. A summary of the primary and secondary outcomes will be captured in CRF 8.

The patient's medical record is a combination of the patient's inpatient paper chart, the inpatient electronic chart in EPIC, and the outpatient electronic chart in OTTR. All of these will be accessed with the minimal use necessary to obtain the information in the eight CRF forms.

VII. 7. Sample Size and Data Analyses

A convenience sample of 50 subjects in total at both sites is the current target enrollment. This is a sufficiently large sample to provide adequate information regarding tolerability to guide future studies, but still allow study completion in a total of 2 years. Participant data will be compared to data obtained from historic controls (1:1 to a maximum of 1:4 ratio of study subjects to controls). Potential controls whose liver donor was part of the intervention arm in a separate clinical trial (the Remote Ischemic Preconditioning in Neurologic Death Organ Donors – RIPNOD) will not be included. Such a comparison would provide additional information regarding safety and preliminary information regarding potential efficacy.

Analyses will include all subjects (except those in whom a transplant did not occur even after enrollment due to donor and other recipient issues) on an intent-to-treat principle. The primary outcome of feasibility will be reported as the proportion of subjects completing all 6 interventions with 95% confidence intervals. In those not completing all 6, the number of interventions per subject will be

reported. Chi-square tests or Fisher's exact tests will be used to assess the significance of the differences in the secondary outcomes of EAD, PRI and AKI, need for renal replacement therapy and complications \geq grade 3b between the RIC and No RIC groups. Differences in length of hospital stay and time to discontinuation of dialysis between the two groups will be compared with either t-test or Mann-Whitney U test. Graft and patient survival data will be compared with Kaplan-Meier estimates. In comparisons of all clinical outcomes p values \leq 0.05 will be considered significant.

VII. 8. Discomforts/Risks and Benefits

Risks: Potential risks to recipients include pain in the lower extremity from application of the tourniquet and ischemic changes in the extremity receiving the intervention. We believe these risks to be minimal based on the following:

- RIC Intervention: Reports in non-operated subjects (healthy volunteers, those with medical conditions and awake preoperative subjects) show that both upper and lower limb RIC intervention is well tolerated from the standpoint of patient's discomfort.^{18,20} To our knowledge, there are no reports in the literature of discontinuation of limb RIC due to patient discomfort/pain. In one study, patients applied RIC in both upper limbs, twice daily, for one year with no mention of withdrawals.²⁴ We will monitor the subject's pain response during the intervention. Last, the patient will have the option to discontinue the intervention at any point if they are unable to tolerate the pain.
- With regards to ischemic changes secondary to tourniquet inflation, the patients at highest risk of this complication (those with pre-existing peripheral vascular disease) are excluded from this study. Otherwise, several clinical studies have demonstrated the safety and feasibility of RIC and none have noted any damage secondary to RIC.^{18,19,25}

Benefits: potential benefits to subjects include improved liver, lung, and kidney function in the immediate post-operative period. These benefits could potentially decrease overall complications, shorten length of stay, and decrease overall morbidity. To our knowledge, there are no published studies or on-going studies (Clinicaltrials.gov and ISRCTN) on the feasibility of applying RIC in liver transplant recipients both during and after surgery. Therefore, the proposed clinical trial would likely extend the knowledge base of RIC in liver transplantation and in postoperative settings. Findings from this study are likely to be useful in planning additional trials in liver and in all other solid organ recipients and in patients undergoing major surgery.

VII. 9. Adverse Events

Adverse events will be tracked for the first 30 days. These include lasting ischemic changes to the lower extremity, pain requiring pain medication, paresthesias, rash related to the tourniquet, or other symptoms that arise and are seemingly related to the intervention.

References

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Appendix A – List of drugs that would preclude enrollment in this study

Sulfonylurea anti-diabetic agents

- Acetohexamide
- Carbutamide
- Chlorpropamide
- Glibenclamide (also known as glyburide)
- Glibornuride
- Gliclazide
- Glycophyramide
- Glimepiride
- Glipizide
- Gliquidone
- Glisoxepide
- Tolazamide
- Tolbutamide

Nitrates

- Nitroglycerine (glyceryl trinitrate)
- Isosorbide mononitrate and dinitrate
- Itramin
- Pentaerythrityl tetranitrate
- Propyl nitrate
- Tenitramine
- Trolnitrate
- Nicorandil

Appendix B – Glossary of Terms

Acute Kidney Injury (AKI): renal impairment above the patient's baseline

- stage 2 AKI:
 - o 2.0-2.9 fold rise in serum creatinine from baseline, or
- Stage 3 AKI:
 - o ≥ 3.0 fold rise in serum creatinine from baseline, or
 - o Serum creatinine of ≥ 4.0 mg/dL, with an acute (< 48 hours) increase of 0.3 mg/dL in serum creatinine or subacute (< 7 days) increase in serum creatinine of 0.5 mg/dL, or
 - o Initiation of renal replacement therapy.
- Many patients will have known pre-existing renal dysfunction prior to transplantation. Of note, patients already receiving renal replacement therapy at the time of transplantation are, by definition, already in stage 3 AKI, and would be excluded from analysis of this portion of the primary outcome. Most patients with pre-operative creatinine of 3.6 mg/dL or greater would also likely have stage 3 AKI prior to transplant (or may erroneously be classified as having developed stage 3 AKI with only a minimal change in creatinine), and will also be excluded from AKI analysis. Urine output criteria for the assessment of AKI will not be used due to the poor reliability of urine output measurement at our institution.

Baseline creatinine: the serum creatinine (mg/dL) obtained immediately prior to transplantation.

Dialysis: any form of renal replacement therapy (see below)

Early allograft dysfunction (EAD): one or more of the following:

- Aspartate Transaminase (AST) or Alanine Transaminase (ALT) $> 2,000$ U/L at any point within the first seven post-transplant days, or
- Total Bilirubin (TB) ≥ 10 mg/dL on postoperative day 7, or
- International Normalized Ratio (INR) ≥ 1.6 on postoperative day 7.

Enrollment: A patient is “enrolled” if they have previously been recruited for study participation (see below) and subsequently undergo orthotopic liver transplant with at least 1 RIC intervention.

Post-operative (post-op) day: the number of calendar days (starting at 12am through 11:59pm) that have elapsed since the time of incision close from the initial liver transplant.

Post-op day 0: the calendar date of incision close of the initial liver transplant.

Prolonged Respiratory Insufficiency (PRI): one or more of the following:

- o ventilator support for >2 days after end of transplant (calendar days starting at post-op day 0), or
- o reintubation after extubation, within 7 days of transplant.

Patients who require brief re-intubation for an endoscopic, radiologic, or surgical procedure would not be considered to have PRI if they are extubated within 2 days of the end of the procedure.

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The re-intubation for PRI is defined as re-intubation required specifically for respiratory distress.

Recruitment: A patient is “recruited” if they have been screened for study participation, found to satisfy all inclusion criteria and none of the exclusion criteria, and sign (or have a legally authorized representative sign) informed consent for study participation.

Renal Replacement Therapy: the use of any of the following:

- slow continuous ultrafiltration (SCUF)
- continuous veno-venous hemofiltration (CVVH)
- continuous veno-venous hemodialysis (CVVHD)
- continuous veno-venous HemoDiaFiltration (CVVHDF)
- intermittent hemodialysis (HD)

Appendix C – Detailed Description of Procedures For Rutgers University – University Hospital

I. MATERIALS:

1. (1) Pneumatic Tourniquet Pumps (PTSii monitors, Delfi Medical Innovations Inc, Vancouver, Canada)
2. (1) Thigh Cuffs
3. 1 Pole mount for the Pumps (These items are stored in a blue plastic bag)
4. IRB approval letter
5. Study protocol
6. CRF 7 (2 pages)
7. Non-invasive Doppler machine with probe and lubricant

II. PROCEDURES

The liver transplant coordinators have standing instructions to call the study coordinator whenever a liver transplant recipient at Rutgers University – University Hospital has a potential donor. The donor will be assessed by standard criteria by the liver transplant team. If the decision is made to proceed with surgery, the patient will be admitted to University Hospital and the study team will be notified. Ask the transplant coordinator about a potential time for initiation of surgery (OR time), if one is available.

I. Subject Recruitment

- a. The study team will be notified of potential subjects for this study either by transplant coordinators and/or transplant surgeons based on acceptance for liver transplant listing. Subjects will be screened. Potentially eligible subjects or their surrogates will be approached either in the out-patient setting (ACC-Transplant clinic at D level or DOC-Transplant clinic suite 7100) or in an in-patient setting at UH for willingness to participate.

Informed consent will be obtained from subjects or their surrogates. A duly completed consent document will be stored in MSB G-501 and a copy provided to the patient/surrogate.

II. Pre-Operative Preparation

- a. Once notified of a potential transplant recipient, contact the admitting transplant team to confirm inclusion and exclusion criteria for the study. A transplant resident (or covering resident, at night and on weekends) is always in the hospital. If the patient is noted not to fall under ALL inclusion criteria, or if ANY exclusion criteria are met, the patient cannot be included in the study, and no further action is required.
 - i. The transplant resident, or covering resident, can be reached at the following pager: 973-312-3984. Dial this number, then after the prompt dial your call-back number, then press #. Wait for all the tones to stop prior to hanging up in order to

ensure the page went through.

- b. If all inclusion criteria are met and none of the exclusion criteria are met, as reported by the covering resident, proceed to University Hospital. The address is:
150 Bergen Street
Newark, NJ 07103
Directions and parking information can be obtained on the University Hospital website at <http://www.uhnj.org/directions/index.htm>.
- c. Once at University Hospital, locate the patient. The patient's location can be obtained ahead of time from the transplant resident. Most patients will be admitted to the F-Yellow floor in the main University Hospital building. The direct phone number for F-Yellow is 973-972-5627. You can ask the security guard for specific directions once entering the main entrance.
- d. Once you have located the patient, ask the patient's nurse if there is anything pending for the patient in terms of pre-operative workup, such as blood draws, EKG, chest X-ray, etc. Do not delay these patient care items for any reason as delaying them could delay the start of surgery.
- e. If the subject had not been approached regarding this trial before, discuss informed consent in detail with the patient. If the patient is moribund or otherwise unable to provide informed consent themselves, discuss informed consent with the legally authorized representative/surrogate. This is usually the same person who signs informed consent for the transplant itself. Obtain signed informed consent from the patient or the next-of-kin and provide a copy of the consent form to the patient and their family.
 - i. While interviewing the patient for potential study enrollment, verify the inclusion and exclusion criteria both with the patient and with the patient's electronic medical record.
- f. **Enroll the subject ONLY AFTER A DECISION HAS BEEN MADE TO PROCEED WITH TRANSPLANT AND WHEN BEING TAKEN TO THE OPERATING ROOM. Sometimes, a potential recipient may be admitted for a transplant but due to donor liver quality and other issues, the transplant may be cancelled. By enrolling subjects earlier than on their way to OR, the likelihood of enrolling subjects but not staying in the study will increase.**

III. Operating Room Preparation

- a. Prior to initiation of surgery, contact the operating room (OR) front desk to confirm the OR time, attending surgeon, attending anesthesiologist, anesthesia resident, and nursing staff. Ask which operating room the surgery will take place in. All liver transplants take place in either OR 4, OR 5, or OR 7. The operating rooms are located in the main University Hospital building on E-Orange.
 - i. The phone number for the OR front desk is 973-972-6901.
 - ii. The phone number for OR 4 is 973-972-5618.
 - iii. The phone number for OR 5 is 973-972-2538.
 - iv. The phone number for OR 7 is 973-972-5617.
- b. Introduce yourself to the OR circulating and scrub nurses, as well as the anesthesia staff. Briefly explain what the study is and what you will be doing. Have the duly completed

informed consent document for that subject, study protocols, and IRB approval with you to show the OR staff.

- e. Request extra towels or a plastic cover (such as a small clean garbage bag) to cover the tourniquet to prevent it from getting soiled.
- d. Ask for an empty IV pole to mount the PTSii monitor.
- e. Once the patient arrives in the operating room and after the patient is intubated, obtain and record a blood pressure reading on the anesthesia monitors. Power on the PTSii monitor, set the timer to 5 minutes, and set the inflation pressure to 250mmHg.
- f. Apply the tourniquet to mid-thigh on the LEFT, halfway between the hip and the knee. Place extra towels or a plastic cover to keep the tourniquet and other equipment from getting soiled.
- g. Connect the rubber tube from the tourniquet to the PTSii monitor.

IV. Intra-Operative Interventions 1 and 2

- a. As you perform the first intervention, educate the anesthesia staff as to how to perform the second intervention, as they will have to perform it at the conclusion of the transplant.
- b. Press the inflate button on the PTSii and note the time of INITIATION of intervention in CRF 7. Fill out the remaining pertinent portions of CRF 7 at this time as well.
- c. During the first cycle of inflation, verify using the Doppler that the patient's dorsalis pedis and posterior tibial pulses are absent. If they are not, gradually increase the tourniquet pressure until they disappear. The maximum allowable pressure is 300 mmHg. Record the actual pressure used in CRF 7.
- d. After 5 minutes, the machine should emit a high pitched beep. IT WILL NOT DEFLATE ON ITS OWN. Press the Deflate button. Make sure the tourniquet deflates. Leave it deflated for exactly 5 minutes. This completes one cycle.
- e. Then press inflate button again leaving the timer on at 5 min. Repeat the above process for a total of 3 cycles of 5 minutes of inflation followed by 5 minutes of deflation.
- f. From the initial inflation to the final deflation will take 25 minutes.
- g. Leave the equipment in place (i.e. leave the tourniquet on the left thigh) for the second intervention, which will take place at the conclusion of the procedure. Work with the anesthesia team to place the equipment in a convenient location to be reached without potentially contaminating the surgical field.
- h. Give CRF 7 to the anesthesia team as they will need to fill out the information related to the second intervention.
- i. At the conclusion of the procedure, the anesthesia team should initiate the second intervention. This should be performed anytime at the initiation of skin closure (in the operating room). If possible, call the operating room at this time for an update as to the operation and to remind the anesthesia team that the next intervention should be started.
- j. Once the operation and second intervention are complete, if you are on campus, gather the supplies. Clean the tourniquet and wash with detergent and water once in the lab. Thank the anesthesiologist, the surgeon, and the nursing staff. You may return to the lab.
 - i. If the operation concludes late at night or you are otherwise occupied, ask the anesthesia or nursing staff to collect the materials for you and leave them in a

pre-determined location for later pickup. Make sure to collect CRF 7 back from the anesthesia team.

V. Post-Operative Interventions

- a. After surgery, most patients are transferred to the Surgical Intensive Care Unit (SICU). Occasionally, if no SICU beds are available, a patient may be transferred to the Post-Anesthesia Care Unit (PACU, or Recovery). Both the SICU and the PACU are located on E-Green in the University Hospital main building.
 - i. The phone number for the SICU is 973-972-5754.
 - ii. The phone number for the PACU is 973-972-5751.
- b. Additional interventions identical to those described above will occur on the 1st through 4th post-operative days between 8am and 10am.
 - i. Post-operative day 0 is defined as the calendar day of skin closure from the initial operation. Post-operative days 1-4 are the subsequent 4 calendar days.
- c. Prior to the intervention, find the patient and introduce yourself to their nurse. Have the duly completed informed consent document for that subject and the IRB approval available to show to the nursing staff.
- d. Repeat 3 cycles of inflation/deflation as described above in section IVb-f, with the exception that the tourniquet pressure used should be 50 mmHg above the patient's current systolic blood pressure. Make sure to fill out CRF 7 while the interventions are in progress.
 - i. The first and third post-operative interventions should be applied to the RIGHT thigh. The second and fourth post-operative interventions should be applied to the LEFT thigh.
- e. If the patient is extubated and able to communicate, ask the patient how much pain they are having IN THEIR THIGH/LEG at the end of the RIC interventions. Record the highest response in CRF 7. Do not include pain scores related to the abdomen or any other source during this data collection process.
- f. Once the interventions are completed, thank the patient and the nursing staff, clean the equipment as described above, and return to the lab to file the paperwork.
- g. If the patient is discharged, dies, or declines further interventions before completion of the fourth post-operative intervention, state that the missing intervention(s) could not be completed and include a brief description as to why on CRF 7.
- h. If at any time a patient is refusing further RIC interventions, do not perform any further interventions as per the patient's wishes. They may still be included in the study for analysis purposes unless they explicitly withdraw consent for use of data.

VI. Surrogate Consent and Decision Making Capacity

- a. If a patient was unable to provide informed consent prior to enrollment in the study, and was instead enrolled by a surrogate, a continuous assessment of patient's decision making capacity should be performed while the patient is admitted to the hospital.
- b. Contact the transplant surgery team every 1-2 days to discuss if the patient has regained decision-making capacity. If they have, fill out a surrogate consent form with them.
- c. If the patient is discharged or dies before regaining decision-making capacity, they will

be included in the study analysis under surrogate consent and no further re-assessment is required.

VII. Other Data Collection

- a. After screening a patient for recruitment, recruiting a patient, or enrolling a patient, be sure to fill out a corresponding section of the enrollment tracking spreadsheet.
- b. The remaining data from CRF 1-8 are best collected by accessing the patient's medical record. Medical records will be obtained from the patient's chart in EPIC at University Hospital as well as the outpatient transplant tracking system OTTR. A study investigator with access to the medical records will be required to fill these out.
- c. Some data from CRF 2 regarding donor information will have to be obtained from the OPO. The OPO for University Hospital is the New Jersey Sharing Network. The phone number for the Sharing Network is 1-800-541-0075.
- d. Some data from CRF 3 will need to be obtained directly from the anesthesia record. At University Hospital, this information is kept in the patient's paper chart for the duration of their hospital stay. Paper charts are located at the nursing stations in the unit where the patient resides. After discharge, the paper charts are transferred to the medical records office.
 - i. The phone number for the medical records office is 973-972-5608.
 - ii. The medical records office is located in the main hospital building, B-level, room B417.